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Modulation by CpG DNA

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13. ABSTRACT (Maximum 200 Words) Breast cancer is the most common non-skin cancer in women, and the American Cancer Society estimates that there will be 203,500 new cases of invasive breast cancer and 40,000 deaths from metastatic breast cancer (MBC in the U.S. in 2002. Thus, patients with MBC who fail conventional therapies are candidates for clinical trials using novel therapies, including immunotherapy. Dendritic cells (DC) are potent antigen-presenting cells that prime antitumor cytotoxic T lymphocytes against tumor-associated antigens and bacterial DNA oligodeoxynucleotides containing unmethylated CpG sequences (CpG DNA) further augment the immune priming functions of DCs. We hypothesize that CpG DNA-stimulated DCs will prime a more potent anti-tumor immune response than non-stimulated DCs. In Year 1 of this proposal, our 2 specific aims are 1) to study the mechanism of antitumor immunity mediated by the vaccination of TS/A mammary tumor-bearing BALB/c mice with CpG DNA-stimulated DCs primed in vitro with necrotic TS/A cells and 2) to determine optimal conditions for CpG DNA stimulation and tumor priming of human DCs.				
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I. INTRODUCTION

Dendritic cells (DC) are potent antigen-presenting cells that prime antitumor immunity against tumor-associated antigens by cytotoxic T lymphocytes, and bacterial DNA oligodeoxynucleotides containing unmethylated CpG sequences (CpG DNA) can further amplify the immunostimulatory capacity of DCs. In this proposal we hypothesize that CpG DNA-stimulated DCs will prime a more potent anti-tumor immune response than non-stimulated DCs. In Aim 1 of this proposal, we test this hypothesis in a pre-clinical model of breast cancer using an established mammary tumor (TS/A) in syngeneic BALB/c mice. DCs are primed by necrotic tumor cells *in vitro* and then injected subcutaneously with CpG DNA in TS/A tumor-bearing mice. In Aim 2, as a prelude to a future clinical trial, we determine the optimal conditions for tumor priming and CpG DNA stimulation of human DCs. In this aim, we will develop optimal loading conditions of human DCs with tumor lysate derived from the MCF-7 human breast cancer cell line.

II. BODY

Task 1. To study the mechanism of antitumor immunity mediated by the vaccination of TS/A tumor-bearing mice with CpG DNA-stimulated DCs primed in vitro with necrotic TS/A cells (Year 1).

Two independent experiments have been undertaken where 55 mice were treated in 9 groups - 5 mice/group for treatment groups 1-7, and 10 mice/group for treatments 8 and 9. The 9 groups of BALB/c mice were injected subcutaneously in the left flank with 10^5 TS/A tumor cells and the tumors were allowed to grow until a nodule was palpable. While tumors were growing *in vivo*, DCs were loaded *in vitro* with necrotic TS/A cells. Necrotic cells were generated by repeat cycles of freezing and thawing, followed by co-culture with DCs for 24 hours. The 9 groups of mice were then injected in the right flank, and boosted one week later, with either (1) PBS, (2) CpG DNA (5'-TCCATGACGTTCTGATGCT-3'), (3) non-CpG DNA (5'-TCCATGAGCTTCCTGATGCT-3'), (4) non tumor-primed DCs (DC^{NP}) (10^6 DCs), (5) tumor-primed DCs (DC^P), (6) DC^{NP} + non-CpG DNA, (7) DC^{NP} + CpG DNA, (8) DC^P + non-CpG DNA, or (9) DC^P + CpG DNA, respectively. We used approximately 5×10^5 DCs and $0.1 \mu M$ CpG DNA in our injections. Tumor size was then measured in a blinded fashion. Unfortunately, in neither of these 2 experiments did we notice a significant reduction in tumor growth in mice treated with DC^P and CpG DNA when compared to control groups. We are therefore in the process of repeating this experiment using a higher number of DCs and a larger quantity ($1 \mu M$ and, if necessary, $10 \mu M$) of CpG DNA.

Task 2. To determine optimal conditions for tumor priming and CpG DNA stimulation of human DCs (Year 1).

A subset of human DCs responsive to CpG DNA has been identified by Krug et al (1). These are plasmacytoid DC (PDC, CD123+) that express the Toll-like receptor-9 (TLR9) which is involved in the recognition of CpG motifs. In particular, a 24-mer CpG ODN (5'-TCG TCG TTT TGT CGT TTT GTC GTT-3') has been tested in these PDC. The authors showed that this CpG ODN-activated PDC stimulates the proliferation of naive allogeneic CD4 T cells, but Th1

polarization requires simultaneous activation of PDC by both CD40 ligation and CpG ODN stimulation. We have undertaken preliminary studies to reproduce these data and, in particular, to establish optimal tumor loading conditions of PDC with tumor lysate from MCF-7, and the studies are in progress. However, these studies are time-consuming in the sense that PDC have to be isolated from PBMC and selected on special BDCA-4 selection columns (Miltenyi), where the yield of PDC is low ($\sim 3 \times 10^6$ cells /buffy coat). Furthermore, these PDC will require activation with both CpG DNA and CD40L *in vitro* prior to administration to patients with metastatic breast cancer, which will not only increase costs but will also complicate IND petitions to the FDA. Recently, however, Gursel et al (2) have identified another CpG ODN sequence (5'-GGT GCA TCG ATG CAG GGG GG-3') that is added to whole PBMC cultures to stimulate the maturation of functional DCs *in vitro* without the addition of other stimulatory agents such as CD40L or the column-selection of PDCs. Because this system is simpler, less expensive, and more easily adaptable to our future clinical trial, we have decided to refocus on this approach to fulfill our stated objective in this aim.

III. KEY RESEARCH ACCOMPLISHMENTS

In the first year of this grant, we have been fine-tuning the optimal experimental conditions for both our *in vivo* (Aim 1) and *in vitro* (Aim 2) studies. With respect to Aim 1, we are confident that we will identify the optimal dose of DCs and CpG DNA to induce the regression of established TS/A tumors in our BALB/c mice. With respect to Aim 2, we are encouraged and excited that a CpG sequence has been characterized that will simplify our ability to not only stimulate the production of functional DCs but to ultimately administer these DCs to patients with metastatic breast cancer.

IV. REPORTABLE OUTCOMES. Not applicable.

V. CONCLUSIONS

In conclusion, in Aim 1, we are in the process of identifying the optimal dose of DCs and CpG DNA to induce the regression of established TS/A tumors in our BALB/c mice. With respect to Aim 2, we will test a new CpG ODN in order to generate functional DCs from PBMC and to optimize loading of these DCs with lysates from MCF-7 human breast cancer cells in order to induce a Th1 response in naïve T cells. With respect to the "so what" implications of this research, it is hoped that these studies will provide a novel and non-toxic immunotherapeutic approach to the treatment of women with metastatic breast cancer.

VI. REFERENCES

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